UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

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MEMORANDUM

DATE: July 22, 2008

SUBJECT: Response to Registrant Comments on the Data Evaluation Record for MRID 46315401: "Ethoprop - Chronic Toxicity to the Sheepshead Minnow (*Cyprinodon variegatus*) During a Full Life-Cycle Exposure."

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The initial Data Evaluation Record (DER) for MRID 46315401,"Ethoprop - Chronic Toxicity to the Sheepshead Minnow (*Cyprinodon variegatus*) During a Full Life-Cycle Exposure," classified the study as **INVALID** because it was performed under conditions that deviated so significantly from recommended protocols. Subsequently, the DER stated that a repeat of the study was warranted because the study did not fulfill guideline requirements (§72-5) for an estuarine/marine fish full life-cycle toxicity test.

In response, Bayer CropScience submitted its comments on the cited deviations and requested that the Agency upgrade the study classification (refer to MRID 47324301 for details) and waive the request for a repeat of the study. However, after submission of the registrant's concerns to

the Environmental Fate and Effects Division's (EFED) Aquatic Biology Technology Team (ABTT) for review and careful consideration of the registrant's position, the Agency has determined that there is insufficient evidence for re-classifying the study and reasserts that the study's deviations from well-established Agency protocols render its results inadequate for risk assessment purposes. Although the totality of the study and its deviations were considered, the two most significant and irreparable deviations contributing to the ABTT's decision to support the INVALID classification of the study were an insufficient number of replicates (two were employed when at least four should be) and poor control performance in hatching success (guidelines specify that hatching success of the controls should be >75%; hatching success was 57% in this study); as outlined in the DER, these deviations compromise the ability of this study to detect significant treatment-related effects. Therefore, EFED still recommends that a new full life-cycle study establishing a reliable NOAEC for estuarine/marine fish be conducted.

Specifically regarding the number of required replicates, the registrant asserted that the guidance and associated feedback from the Environmental Protection Agency (EPA) has been inconsistent. However, the ABTT would like to note that the Agency's Office of Pesticide Programs' (OPP) existing Standard Evaluation Procedures (SEPs) for fish life-cycle toxicity tests clearly state that these tests should be initiated with four replicates.

One source of confusion regarding the required number of replicates may stem from the harmonization of testing guidance and requirements of the EPA's Office of Pollution, Prevention, and Toxics (OPPT) and OPP in the Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 850 series for Ecological Effects Test Guidelines. For example, OPPTS guideline 850.1500 for fish life-cycle toxicity tests presents no guidance on the number of replicates, and while OPPTS guideline 850.1400 for fish early life-stage toxicity studies does not require a set number of replicates, it does state that "at least two replicate test chambers" be employed. The flexibility allowed for under these guidelines was designed to accommodate both the needs of OPPT and OPP in toxicity testing.

The use of less than four replicates is only allowed for OPPT purposes and any others when the primary goal of testing is to solely establish regression-based estimates of toxicity (e.g., LC_{50}). As generally understood and outlined in the fish life-cycle and early life-stage toxicity test SEPs designed specifically for OPP's purposes, when establishing hypothesis or analysis of variance-based endpoints (i.e., NOECs and LOECs), tests should be initiated with at least four replicates in order to adequately account for variability. The pitfalls associated with having too few replicates when performing hypothesis-based testing is illustrated by the low detectable minimum significant difference (MSD) for a number of endpoints in the study currently under review (MSD as expressed in % difference from control ranged from 2.9 to 62.6 for all endpoints). To avoid future confusion, new OPPTS 850 guidelines for both fish early life-stage and life-cycle studies that are currently being finalized will be made to be consistent with the existing SEPs and will clearly distinguish the different needs of OPPT and OPP regarding the appropriate number of replicates to be used in testing.

With regards to poor control performance in hatching success, the registrant's main argument is that it is "the adjusted hatching success that is required to be >75% in controls" and that this is clearly

laid out in OPPTS 850.1400 which specifies that "overall survival of **fertilized** eggs in the controls must be greater than or equal to the limits defined" in the guideline (i.e., >75%) (emphasis added). Subsequently, the registrant adjusted hatching success based on viability estimates after the test was completed to account for fertility, and argued that the adjusted hatching success meets the control performance standards for hatching success (>75%). However, the Agency would like to indicate the guideline protocol specifies that the "test is begun by placing **fertilized** eggs in the test chambers (page 1) (emphasis added)," and the ASTM referenced by this guideline outlines a method for confirming fertilization for sheepshead minnow prior to test initiation. Therefore, only fertilized eggs are to be selected for use in this test and no post hoc adjustment should need to be made to account for low fertility rates if the test is conducted properly.

American Society for Testing and Material (ASTM). Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes. ASTM E 1241-92, p. 180-207, Philadelphia, PA (1992).

Rexrode, M. and T.M. Armitage. 1986. Standard Evaluation Procedure; Fish Life-Cycle Toxicity Tests. Hazard Evaluation Division, Office of Pesticide Programs. Washington, D.C. 20460. EPA 540/9-86-137.

U.S. Environmental Protection Agency. 1996. *Ecological Effects Test Guidelines, OPPTS 850.1400: Fish Early-Life Stage Toxicity Test*, (Public Draft), Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C.